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Study of Koi Fish (*Cyprinus carpio* Linn.) Chromosome Using Chromosome Image Analyzing System

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Abstract. Koi fish (*Cyprinus carpio* Linn.) is an ornamental fish with high economic value and has long been cultured in Indonesia. Koi fish has a uniqueness appeared from its phenotype and wide intraspecies genetic variation. Genetic analysis i.e. cytogenetics is important to gain information about species relatedness, chromosomal abnormalities, and further lead to the fish culture development. Koi fish chromosome information in Indonesia has not been reported so far. Therefore the aim of this study was to obtain information about the chromosomes of Kohaku koi fish in Indonesia. In addition, in this study, we applied CHIAS (*Chromosome Image Analyzing System*) software to study koi fish chromosome karyotyping for the first time. The cells were cultured using Roswell Park Memorial Institute medium at a temperature of 37 °C in a 5 % CO₂ incubator. The cell synchronization at metaphase was carried out by two-hours colcemid treatment. The samples were then subjected to a hypotonic solution and fixed with a solution of methanol and glacial acetic acid. According to the results, koi fish chromosome number ranged from $2n = 100$ to $2n = 102$. Furthermore, koi fish chromosomes could successfully be arranged based on their size by using CHIAS. In conclusion, koi fish chromosome numbers were varied and CHIAS has been proven to be powerful to analyze the small size and the high number of chromosome. Results of our study could further be applied for other koi chromosome researches as well as for the development of cytogenetics in Indonesia.

Keywords: CHIAS, chromosome, culture cell, *Cyprinus carpio*, koi fish

INTRODUCTION

Indonesia has a large biodiversity including the diversity of freshwater ornamental fish species. One of the freshwater ornamental fish commodities that became the most favorable in national and international markets is koi fish (*Cyprinus carpio* L.) [1]. Koi fish has long been domesticated and cultured due to its phenotypic variation [2]. This fish also belongs to the type of carp that has a high economic value and has a stable selling value fluctuation in the market [3, 4]. Research for the development of koi fish culture needs to be done to support the increase of koi fish production [1]. The availability of genetic information such as sophisticated chromosome analysis [5] is important for the development of koi fish cultivation and conservation. However, to date, there has been no report on the chromosome of koi fish in Indonesia.

Cytogenetics including chromosome analysis is essential to study the diversity, relatedness, and further lead to the conservation effort of the species. One of the basic information is the karyotype of the chromosome where the chromosome number, structure, and arrangement are presented [6]. Nevertheless, karyotyping is somehow challenging if it is carried out manually, especially when the small size and high number chromosomes are studied.

Chromosome Image Analyzing System (CHIAS) is a Java-based image analyzing software that has been developed to specifically analyze the chromosome including basic information (number, size, structure), centromeric detection, banding analyzes, up to the automated karyotyping of the studied-chromosome [7]. Previous studies

reported the usefulness of CHIAS for chromosome analyzes, however, the application of CHIAS is still limited mostly to the plant chromosomes such as *Indica rice* [7] and *Trifolium pratense* L [8] which has a relatively small number of chromosomes ($2n = 24$ and $2n = 14$ respectively). There has been no report so far on the koi fish chromosome analysis using CHIAS with the high number of the chromosomes ($2n = 100-102$) which are prone to overlapping. The application of CHIAS would be beneficial for koi fish chromosomes analysis due to the small size and high number of chromosomes. This study is conducted to analyze the chromosomes of Kohaku koi fish (*C. carpio* L.) and evaluate the advantage of CHIAS for the koi fish chromosome analysis. The results are expected to provide the basic chromosome information (number, structure, and arrangement) of Kohaku koi fish that would further be beneficial for the development of koi fish culture and conservation in Indonesia.

MATERIALS AND METHOD

The samples of this study were the certified native offspring of Kohaku Koi fish from Blitar and Kohaku Koi fish from Depok which the offspring was unknown. The method used is based on Howe et al. with modifications [9]. Chromosomes were prepared using the blood culture technique.

The blood samples were taken from the Kohaku koi fish by adding 0.1 mL of sodium citrate 3.8 %. The blood was collected in a heparin tube and then cultured in the medium. The culture medium consisted of RPMI 1640 medium (Gibco®), FBS, PHA-M, and antibiotic-antimycotic. The culture was incubated at 37 °C with 5 % CO₂ for 72 hours. The harvesting of blood cultures was carried out after counting the number of cells with the Countess™ Automated Cell Counter [Invitrogen].

Prior to harvesting, 10 µg/mL colcemid was added to the culture, followed with the incubation for 3 hours at 37 °C. Samples were then centrifuged at 200 x g for 5 minutes, hydrolyzed with a warm 0.075 M KCl at 37 °C and incubated for 8 minutes at 37 °C. After incubation, samples were centrifuged at 200x g for 10 minutes at 25 °C and then fixed with the cold and freshly-prepared methanol:acetic acid (3:1). Following 10 minutes incubation, the samples were centrifuged again at 200 x g for 5 minutes. The staining of chromosomes was carried out with 5 % Giemsa staining solution prior to the observation using inverted microscope. Chromosome images were analyzed using ImageJ and CHIAS software.

RESULTS AND DISCUSSION

The chromosomes of Kohaku koi fish were shown in Fig. 1. The size of the chromosomes was relatively small with a high number (Fig. 1a). Thus, it was difficult to obtain the well-spread koi fish chromosome images. However, the border of each chromosome could be recognized (Fig. 1b) and image analysis was required to enhance the contrast and resolution of the images.

The similar characteristics of the high number and small size of the *C. carpio* L. chromosomes have also been previously reported by Ojima et al. and Arisuryanti et al. [6, 10]. To further analyze the number of chromosomes, the background of the image was removed by using ImageJ software (Fig. 2). The results showed a well-distributed of Kohaku koi metaphase chromosome.

As shown in Fig. 2, the background of the image could be removed by using ImageJ software. The borderlines of every individual chromosome were clearly defined. The figure also depicted that the size of the chromosome varies widely. Some chromosomes showed a bigger size than the others. Some others were relatively small. Further analysis to provide the chromosome arrangement was necessary.

Furthermore, the number of chromosomes have successfully been counted. In this study, we used two types of koi fish samples i.e. from Blitar which was the native offspring of Kohaku koi fish and the fish from Depok which the offspring is unknown. The results showed that the chromosome number of koi fish from Depok were more varied ($2n = 100-102$) than those of the fish from Blitar ($2n = 100-101$). The offspring of the samples might explain this variation. The varied number could be induced by the environmental conditions as a response of adaptation [3]. Furthermore, the unknown native offspring Koi fish from Depok could also be caused by the breeding with other species or a variant of the *C. carpio* L.

According to the results obtained (Table 1), the Kohaku koi fish number was ranging from $2n = 100$ to $2n = 102$. Previous studies have reported the various number of *C. carpio* L. Anjum (2005) revealed that the chromosome

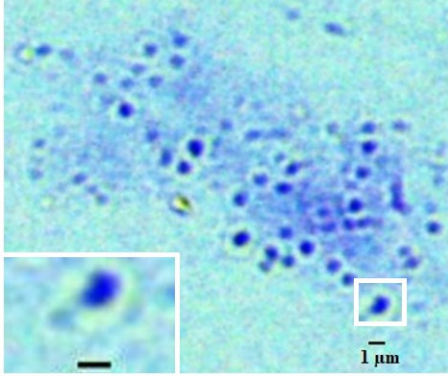


FIGURE 1. Metaphase chromosomes of Kohaku koi fish, where the number of chromosomes is high with the small size. The enlarged representative chromosome.



FIGURE 2. The distribution of Kohaku koi fish metaphase chromosomes after ImageJ processing.

TABLE 1. Number of chromosomes of Kohaku koi fish (*C. carpio* L.).

No.	Koi Kohaku Fish	Chromosomes Amount
1.	Kohaku koi fish from Blitar 1 (*B1) native offspring	2n = 100
2.	Kohaku koi fish from Blitar 2 (*B2) native offspring	2n = 100
3.	Kohaku koi from Depok 1 (*D1) not native offspring	2n = 101
4.	Kohaku koi fish from Depok (*D2) not native offspring	2n = 100
		2n = 101
		2n = 102

(*) Description: In this experiment we use 4 individuals of koi fish, one individuals for each type : B1 & B2 is native offspring of Kohaku koi fish from Blitar; D1 & D2 is not native offspring of Kohaku koi fish from Depok

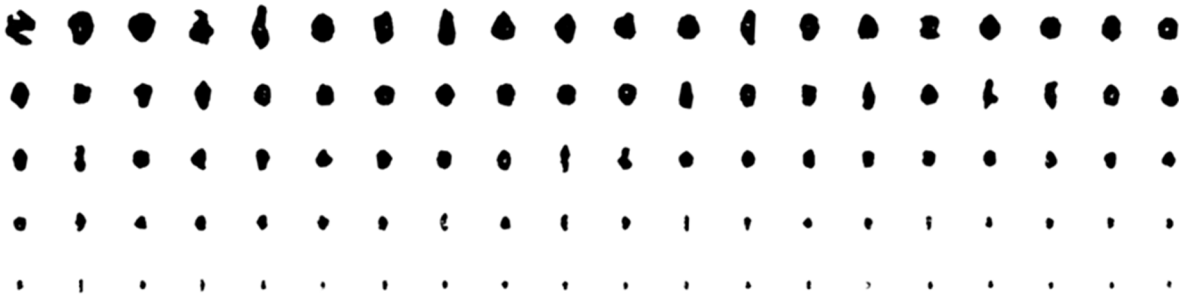


FIGURE 3. The karyotype of Kohaku koi fish chromosomes by using CHIAS (2n = 100).

numbers of wild common carp in Vietnam was $2n = 100$ [11]. Ojima et al. reported that koi fish chromosome number in Japan were around $2n = 100-102$ [10]. Similar results were also reported by Leane [12]. In other studies, Arisuryanti and Arisuryanti et al. found that the chromosome number of Majalaya fish (*C. carpio* L.) were $2n = 96-102$ [6]. Therefore, the chromosome number found in this result is in accordance with previous reports.

To further analyze the number of chromosomes, karyotyping of the chromosomes was carried out using CHIAS (Fig. 3). It is clear, that the chromosomes could successfully be arranged, and the detail of each chromosome could be investigated. Karyotyping also provides information about each chromosome size and structure. The results obtained from this study could be used as the basic information for further exploration of cytogenetic of koi fish. The usefulness

of CHIAS in conjunction with the ImageJ software has also been proven for the analysis of the high number and small size of the chromosomes.

CONCLUSION

The chromosome number of Kohaku koi fish (*C. carpio* L.) were $2n = 100-102$. The Chromosome Image Analyzing System (CHIAS) could be used to evaluate the high number and small size of the chromosomes. The results of our study could be applied for further koi chromosome research as well as for the development of cytogenetics in Indonesia.

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